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IN-VITRO EVALUATION OF FUNGICIDES, PLANT EXTRACTS AND BIO-CONTROLAGENTS AGAINST RICE BLAST PATHOGEN *MAGNAPORTHE ORYZAE* COUCH.

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Abstract

Among 5 fungicides *viz*., Thiophanate-methyl, Carbendazim, Fosetyl-aluminium, Mancozeb and Copper oxychloride, used against the *Magnaporthe oryzae*, only Mancozeb appeared as the highly effective fungicide that completely inhibited the mycelial growth of the fungus. All other fungicides showed little effect at higher concentrations. The extracts of garlic (*Allium sativum* L.), neem (*Azadirachta indica* L.) and calatropis (*Calotropis procera* L.) when used against *M. oryzae* by food poisoning method, only higher dose of garlic completely inhibited the mycelial growth of the test fungus. Six bio-control agents *viz*., *Trichoderma harzianum, Trichoderma polysporum, Trichoderma pseudokoningii, Gliocladium virens, Paecilomyces variotii* and *Paecilomyces lilacinus* were used. Maximum mycelial inhibition of *M. oryzae* was provided by *P. lilacinus* followed by *Trichoderma* spp.

Introduction

Rice (*Oryza sativa* L.) is important staple food and cash crop of the world as well as of Pakistan. Worldwide annual production of rice is 661811 thousand tonnes (Anon., 2009). In Pakistan it contributes 15% to the foreign exchange earnings and accounts 1.3% to GDP (Anon., 2009). In Pakistan rice is grown on an area of 2581.2 thousand hectares with total production of 5438.4 thousand tonnes and average yield is 2107 kg per hectare. Punjab, Sindh and Balochistan are the major rice producing provinces of Pakistan. Rice crop is subjected to attack by 50 diseases include 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Hollier *et al*., 1993; Webster & Gunnell, 1992; Jabeen *et al*., 2011 a & b; Jabeen *et al.*, 2012). However, major diseases are rice blast, brown spot, bacterial leaf blight and leaf streak, sheath blight, sheath rot, *Fusarium* wilt or Bakanae, stem rot, Tungro virus, false smut and post-harvest diseases (Sharma & Bambawale, 2008). It is estimated that about 14-18% yield reduction was caused by these diseases worldwide (Mew & Gonzales, 2002).

Rice blast caused by a filamentous, ascomycete fungus *Magnaporthe oryzae* (syn: *Pyricularia oryzae* Cav.) is an infectious fungal disease which is distributed worldwide and prevailing in more than 85 countries of the world (Gilbert *et al*., 2004; Scardaci *et al*., 1997). Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses in these areas ranging from 50 to 90 % of the expected crop (Agrios, 2005; Mehrotra, 1998; Robert, 1991; Chaudhary *et al*., 1994). Under usual conditions yield losses due to blast ranged from 1-50% in different rice growing regions of the world depending upon the type of cultivars grown and environmental conditions prevailed (Greer *et al*., 1997). Rice blast is also recognized as one of the important disease in Pakistan causing considerable losses in yield (Hafiz, 1986; Shazia *et al*., 2003).

Chemicals are commonly applied for controlling rice blast disease (Oh, 2007; Anwar *et al*., 2002; Gohel *et al*., 2008). However, the frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms, and develop fungicide resistance into the pathogens and residual toxicity in plant parts. On the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on environment (Iftikhar *et al*., 2010; Babar *et al*., 2011). *Trichoderma* spp., the well known antagonistic fungus are widely used in agriculture as biofungicides (Mukherjee *et al*., 2008). *Trichoderma* spp., inhibited the mycelial growth of rice blast fungus (Ouazzani *et al*., 1998). Similarly, plant extracts like garlic juice successfully reduced the infection caused by *Magnaporthe* sp., on rice (Slusarenko *et al*., 2008). Mycelial growth of rice blast fungus was also significantly reduced by water and ethanol leaf extracts, and oil extract of neem seed (Amadioha, 2000). The purpose of this study was to evaluate comparative efficacy of different chemical fungicides, plant extracts and antagonistic fungi against *Magnaporthe oryzae* causing rice blast.

Materials and Methods

Inoculum of test fungus: *M. oryzae* was isolated during previous study (Hajano, 2010). It was isolated from diseased leaves of rice varieties collected from district Badin, Sindh during 2010.

Effect of different fungicides on mycelial growth of *Magnaporthe oryzae*: Five fungicides *viz*., Topsin-M (70% Thiophanate-methyl), Bavistin (50% Carbendazim), Aliette (80% Fosetyl-aluminium), Dithane M-45 (80% Mancozeb) and Copper oxychloride (50% Copper oxychloride) were tested by food poisoning method at 10000 ppm, 1000 ppm and 100 ppm by adding at the time of pouring after sterilization of PDA medium. PDA medium without any fungicide served as control. Before pouring of sterilized medium in Petri dishes streptomycin sulphate at $1mlL^{-1}$ medium and penicillin at 1000,000 $unitsL^{-1}$ were used as antibiotic to avoid bacterial contamination. After solidifying of the medium, one cm

disc of pure culture of test fungus (*M. oryzae*) was placed in the center of Petri dishes and incubated at 30°C. There were five replications of each treatment. Radial mycelial growth of the test fungus was recorded in mm after each 24 hours till the upper surface in control treatment was fully covered with the mycelial growth of the fungus.

Effect of different plant extracts on mycelial growth of *Magnaporthe oryzae*: Fifty gram of neem leaves (*Azadirachta indica* L.), Calatropis leaves (*Calotropis procera* L.) and garlic bulb (*Allium sativum* L.) were washed with tape water and then grinded in pestle mortar. After grinding, strain them through muslin cloth by adding 250 ml of distilled water. The prepared extracts were added at the time of pouring in the sterilized PDA medium at 1, 2 and 4 ml /15ml, separately. Streptomycin sulphate at $1mL^{-1}$ medium and Penicillin at 1000,000 $units L^{-1}$ were used as antibiotic to avoid bacterial contamination. PDA medium without plant extract was kept as control. There were 5 replications of each treatment. After solidifying of culture medium, one cm disc of pure culture of the test fungus was placed in the center of each Petri dish and incubated at 30°C. Diameter of growing colonies of the fungus was recorded in mm after each 24 hour till the plates were filled in either treatment.

Effect of different bio-control agents on mycelial growth of *Magnaporthe oryzae*: Different bio-control agents such as *Trichoderma harzianum, Trichoderma polysporum, Trichoderma pseudokoningii, Gliocladium virens, Paecilomyces variotii* and *Paecilomyces lilacinus* were tested under laboratory conditions against rice blast causing fungus, *Magnaporthe oryzae*. The inoculum of each bio-control agent was obtained from Culture Collection Center, Department of Agriculture, University of Karachi, Karachi. One cm disc of test fungus and bio-control agent were placed at opposite sides to each other in Petri dishes that containing sterilized PDA medium. There were 6 replications of each bio-control agent and were incubated at 30°C. Petri dishes containing the test fungus and bio-control agents separately incubated at 30°C served as control. Colony diameter of both bio-control agent and the test fungus were recorded after each 24 hours by giving straight line in the center of both colonies with permanent marker. The mechanism of antagonism was observed when the colonies of both fungi met. The following interactions between the test fungus and antagonist were noted (Yaqub & Shahzad, 2005).

a) Colonies of bio-control agent and test fungus met each other; the test fungus overgrew the colony of bio-control.
b) Growth of test fungus was inhibited by bio-control agent produced coiling around mycelium of the test fungus.
c) Colonies of bio-control agent and test fungus met each other, no further growth either of the test fungus or the bio-control agent was observed.
d) Colonies of the test fungus and bio-control agent intermingled.

Finally the data was analyzed by ANOVA using Statistic 8.1 software. Least significant differences (LSD) were calculated using significant level at P = 0.05.

Results

Effect of different fungicides on mycelial growth of *Magnaporthe oryzae*: The mycelial growth of *M. oryzae* showed a different trend in response to different fungicides used. The mycelial growth of *M. oryzae* was significantly different within the 3 concentrations of each fungicide (ANOVA: F = 716.45; df = 79; p< 0.05). Generally, among the 5 tested fungicides, only Mancozeb appeared as highly effective fungicide against the *M. oryzae*, growth of the fungus was completely restricted at 1000 and 10,000ppm of Mancozeb. All other fungicides failed to inhibit the mycelial growth of the fungus completely, mycelial growth of the test fungus at higher concentration (10,000ppm) of Thiophanate-methyl was (20.84 mm), Carbendazim (20.66 mm), Fosetyl-aluminium (12.80 mm) and Copper oxychloride (22.16 mm). These fungicides slightly checked the mycelial growth of *M. oryzae* (Fig. 1).

Effect of different plant extracts on mycelial growth of *Magnaporthe oryzae*: Among the 3 plant extracts, garlic extract performed better and significantly inhibited the mycelial growth of *M. oryzae* at its all three concentrations (ANOVA: F = 214.41; df = 49; p< 0.05). The neem extract failed to suppress the mycelial growth of the test fungus at its lower concentrations (Fig. 2). However, the higher concentration (4 ml) of neem extract was highly effective in checking the mycelial growth of *M. oryzae* (20.58 mm) (Fig. 2). The extract of Calatropis at all concentrations failed to inhibit the colony growth of *M. oryzae* (Fig. 2).

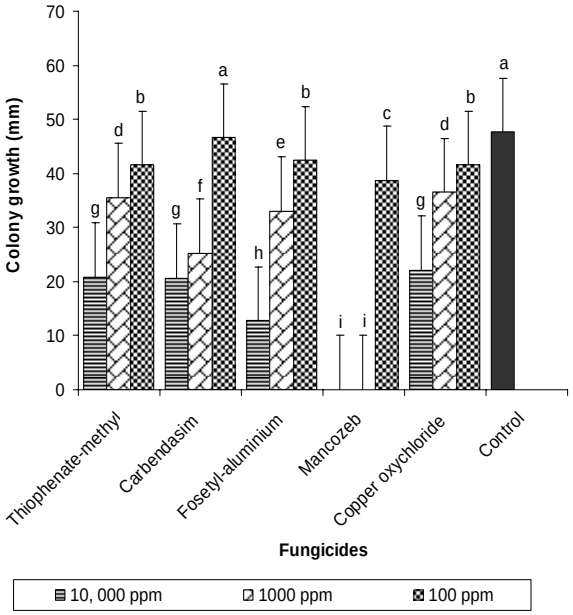

Fig. 1. Effect of different fungicides on mycelial growth of *Magnaporthe oryzae*.

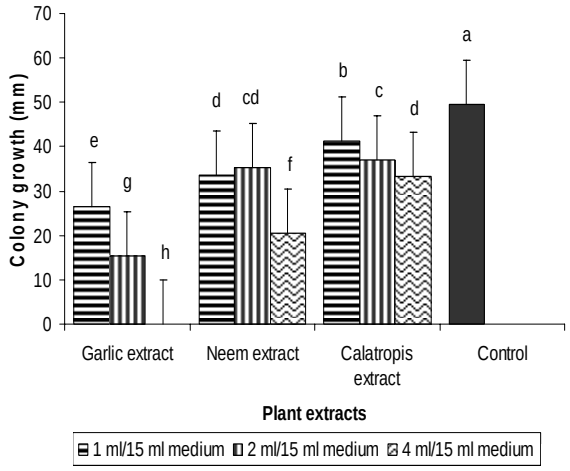

Fig. 2. Effect of different plant extracts on mycelial growth of *Magnaporthe oryzae*.

Effect of different bio-control agents on mycelial growth of *Magnaporthe oryzae*: Six different bio-control agents *viz*., *Trichoderma harzianum*, *T. polysporum*, *T. pseudokoningii*, *Gliocladium virens*, *Paecilomyces variotii* and *P. lilacinus* were evaluated against *M. oryzae* on PDA medium. Maximum inhibition of mycelial growth of *M. oryzae* was observed against *P. lilacinus* (15.60 mm) followed by *T. pseudokoningii* (25.0 mm), *T. polysporum* (30.0 mm) and *T. harzianum* (34.6 mm), respectively. However, the minimum inhibition of mycelial growth of *M. oryzae* was obtained by *P. variotii* (41.4 mm) and *G. virens* (39.2 mm). *T. harzianum*, *T. polysporum* and *T. pseudokoningii*, that showed "D" type interaction, whereas *G. virens*, *P. variotii* and *P. lilacinus* showed "C" type interaction (Table 1), where colonies of bio-control agents and *M. oryzae* met each other, no further growth either of the pathogen or the bio-control agent was observed.

Table 1. Effect of different bio-control agents on mycelial growth of *Magnaporthe oryzae*.

S. No.	Bio-control agent	Incubation days	Diameter of pathogen in interaction (mm)	Diameter of bio-control agent (mm)	Type of interaction
1.	*Trichoderma harzianum*	4	34.600 d	55.4	D
2.	*T. polysporum*	4	30.000 e	60	D
3.	*T. pseudokoningii*	4	25.000 f	65	D
4.	*Gliocladium virens*	6	39.200 c	50.8	C
5.	*Paecilomyces variotii*	4	41.400 b	48.6	C
6.	*P. lilacinus*	5	15.600 g	74.4	C
7.	*M. oryzae* (control)	6	54.200 a	-	-

LSD (0.05%) 0.9142
(ANOVA: F= 1571.92; df= 34; P< 0.05)

Discussion

The colony growth of *M. oryzae* was gradually reduced with increasing concentrations of all the fungicides. Among the fungicides used, Mancozeb completely inhibited growth of the test fungus. Mancozeb appeared to be highly effective fungicide as *M. oryzae* failed to grow at 10,000 and 1000 ppm concentrations, respectively. Different workers studied wide range of fungicides and found many of them effective for rice blast fungus, *M. oryzae* (Mohamed, 1980; Verma *et al*., 1985; Misra & Dharam, 1990; Prajapati *et al*., 2004). Our findings are also in confirmation to those reported by Anwar *et al*., (2002) who observed that Mancozeb exhibited excellent control of rice blast disease caused by *M. oryzae*. Similarly, Gohel *et al*., (2008) evaluated 19 fungicides against *M. oryzae* and found that Tricyclazole, Mancozeb, Carbendazim, Iprobenfos, Propiconazole and Edifenphos were highly effective against the test fungus.

In the present investigation, the extract of garlic, neem and calatropis with three different doses were tested against the causal fungus *M. oryzae*. Only garlic extract at higher dose successfully inhibited the growth of *M. oryzae* on PDA medium. The neem extract at higher dose of 4 ml/15 ml medium provided moderately reduction in the growth as compared to calatropis and the control. Our results are in close agreement to those reported by Punja **(**2005**)** and Slusarenko *et al*., (2008) who found that garlic juice and compound obtain from it (Allicin) were highly effective against rice blast fungus *M. oryzae*. Similarly, Fiona *et al*., (2005) also observed that allicin successfully inhibited the growth and infection of *M. oryzae*. Others like, Amadioha (2000) and Rajappan *et al*., (2001) also reported that neem extracts reduced the mycelial growth of *M. oryzae*.

Among the six bio-control agents tested against the *M. oryzae,* the maximum inhibition was obtained from *P. lilacinus* followed by *T. pseudokoningii*, *T. polysporum* and *T. harzianum.* The use of antagonistic fungi to control the destructive plant pathogens is getting more importance since few decades (Burges & Jones, 1998; Whipps, 2001). Fungal bio-control agents like *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., etc have proved to be effective for controlling numerous destructive plant pathogens (Larena *et al*., 2002; Elad, 2000; Srinon *et al*., 2006; Dawar *et al*., 1993; Yaqub & Shahzad, 2005).

Our results are in accordance to those reported by Gouramanis (1995) who observed that antagonistic such as *T. harzianum* and *Chaetomium globosum* gave 70-88% mycelial and conidial inhibition of *P. oryzae*. Similarly, Watanabe (1985) evaluated different species of *Trichoderma* against 24 airborne plant pathogens including *M. oryzae* and found that *T. hamatun*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* and *T. viride* had strong antagonistic potential. He also found that selective isolates of *T. harzianum* and *T. viride* showed severe antagonism against *M. oryzae*, while *T. polysporum* was weaker antagonist.

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